Bulgarian Chemical Communications, Volume 52, Special Issue D (pp. 203-211) 2020

Interfacial concentrations of catechin in corn oil-in-water emulsions: effects of surfactant concentration, oil-to-water ratio and temperature

N. Martínez-Aranda[1], S. Losada-Barreiro[1,2], C. Bravo-Díaz[1]*

[1]*Universidad de Vigo, Fac. Química, Dpto Química Física, 36200, Vigo, Spain*
[2] *REQUIMTE-LAQV, Departamento de Química e Bioquímica, Faculdade de Ciências. Universidade do Porto. 4169-007, Portugal*

Received: February 07, 2020; Revised: May 04, 2020

Control of the oxidation of lipids in food-grade emulsions by antioxidants (AOs) is challenging because AOs should be present at the site of reaction with the lipid radicals (the interfacial region) in a concentration high enough to make the rate of the inhibition reaction equal to, or higher than, the rate of propagation of lipid radicals. Here we investigated the effects of increasing surfactant volume fraction (Φ_I), oil-to-water ratio, O/W, and temperature (T) on the aqueous and interfacial concentrations of catechin (CAT) in stripped corn oil-in-water emulsions. CAT only distributes between the aqueous and the interfacial region of emulsions and its efficiency depends on the effective concentration in the interfacial region. The partition constant P_W^I values are independent of Φ_I and of the O/W ratio, but incorporation of CAT into the interfacial region increases upon increasing temperature. However, the effective interfacial concentration of CAT decreases upon increasing Φ_I (constant T and O/W) and slightly increases upon increasing T and O/W ratio at constant Φ_I.

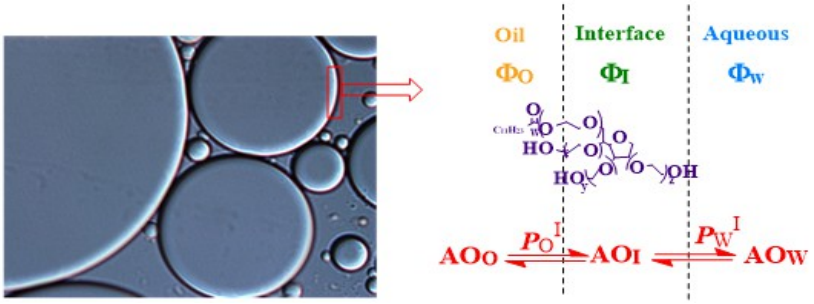
Keywords: Catechin, partitioning, emulsions, interfacial concentrations, corn oil.

INTRODUCTION

Lipids are mostly present in foods as oil-in-water emulsions, and addition of antioxidants (AOs) is one of the main practical technologies employed in the food industry to minimize their oxidation, a radical reaction of great concern because of its close relationship to food quality deterioration and health complications [1-4]. AOs react with lipid peroxyl radicals present in the system, equation (1), yielding a lipid hydroperoxide (LOOH) and a radical antioxidant AO$^\bullet$ much less reactive than the lipid peroxyl radical LOO$^\bullet$[5-7]. AOs are effective in inhibiting the lipid oxidation reaction when the rate of production of peroxyl radicals, r_p, is lower than the rate of the inhibition reaction, r_{inh} [8].

$$LOO^\bullet + AOH \rightarrow LOOH + AO^\bullet \quad (1)$$

Most lipids in foods exist in the form of oil-in-water (O/W) emulsions, consisting of small spherical oil droplets surrounded by an aqueous solution and kinetically stabilized by addition of surfactants, Scheme 1.



Scheme 1. Microphotograph of an oil-in-water emulsion that, conceptually, is divided into three distinct regions, the continuous (aqueous) region, the oil interior and the interfacial region. The scheme on the right shows the partitioning of an antioxidant, AO, of moderate hydrophobicity between those regions is also shown.

The unsaturated components of the lipids are prone to oxidation, and added antioxidants partition between the oil (O), water (W) and interfacial (I) regions of emulsions. Therefore, their efficiency in minimizing lipid oxidation depends not only on the rate constant (k) of the chemical reaction between the AO (AOH in equation (2)) and the peroxyl lipid radical but also on their concentrations at the reaction site, equation 2.

* To whom all correspondence should be sent:
E-mail cbravo@uvigo.es

$$v_{inh} = k_w(LOO^{\bullet}_W)(AOH_W) + k_O(LOO^{\bullet}_O)(AOH_O) + k_I(LOO^{\bullet}_I)(AOH_I) \quad (2)$$

We have recently demonstrated that the interfacial region of the emulsions is the main region where the inhibition reaction takes place [9-12], and thus, we can safely drop off the aqueous and oil contributions to the overall rate of inhibition in equation (2). This means that, once we have chosen an antioxidant of interest, its efficiency depends on its interfacial concentration, the higher the interfacial concentration, the higher the inhibition rate and thus its efficiency.

However, prediction of the interfacial concentrations of antioxidants is not a simple task because the partitioning of the antioxidant strongly depends on the hydrogen-bond ability of the antioxidant and the solvent properties of the various regions. In recent works [9-12], we showed that the interfacial concentration of antioxidants does not correlate with their hydrophobicity, increasing upon increasing the hydrophobicity up to a maximum after which, a further increase in the AO hydrophobicity results in a decrease in its interfacial concentration. This parabolic-like variation is known as the "cut-off" effect, Figure 1, and is a consequence of the differential solubility of the antioxidant in the oil and interfacial regions of the emulsion. This parabolic variation for the interfacial concentration of antioxidants, however, correlates with their antioxidant efficiency.

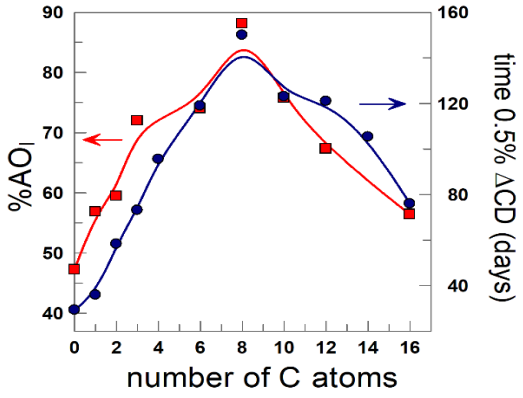
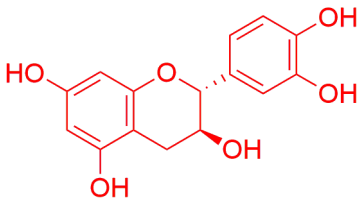


Figure 1. A parallel and parabolic variation in interfacial concentrations of hydroxytyrosol derivatives and in their antioxidant efficiencies in 4:6 olive oil-in-water emulsions. Adapted from [11], copyright Americal Chemical Society.

Thus, proper understanding on how antioxidants are distributed in emulsified systems is important to predict their efficiency and to the food industry for improving the quality and shelf-life of lipid-based products [13].

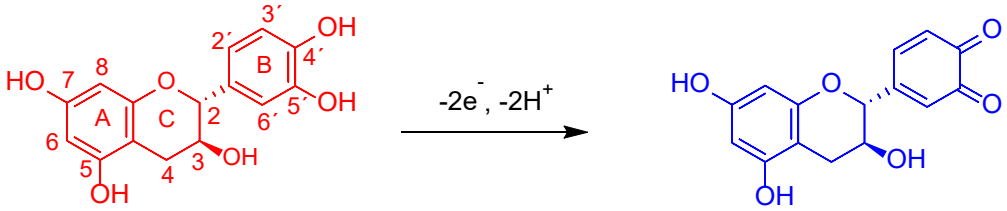
However, the physical impossibility of separating the interfacial region from the oil or aqueous regions makes the prediction of the distribution of antioxidants in emulsified systems a challenge [14]. In general, two partition constants are needed to describe the distribution of antioxidant, that between the oil-interfacial (P_O^I) and that between the aqueous-interfacial regions (P_W^I). These partition constants can not be measured independently by isolating and analyzing the concentration of antioxidant in each region because of the emulsion breakdown and thus, determining antioxidant distribution in emulsified systems requires determining partition constants in the intact emulsion [14]. Here we have employed our kinetic methodology to investigate the effects of surfactant concentration (Φ_I), oil-to-water ratio (O/W) and temperature (T) on the interfacial concentrations of (+) catechin, CAT, Scheme 2. The health benefits of the dietary intake of flavonoids make catechins (flavan-3-ol derivatives) the center of many nutritional studies because of their antioxidant properties [15-17].



(+)-catechin (CAT)

Scheme 2. Chemical structure of catechin.

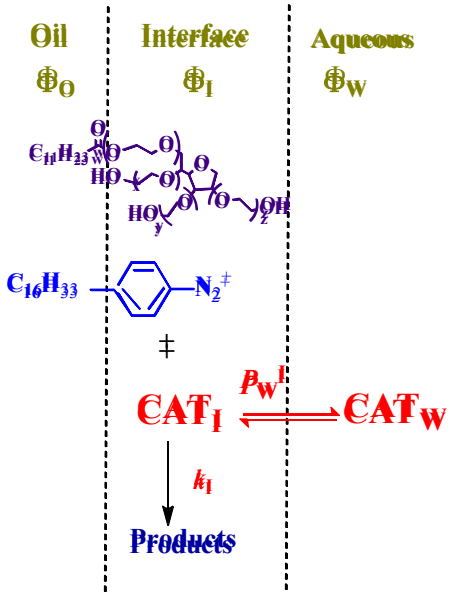
The specific mechanism by which CAT reacts with lipid radicals has not been fully stablished so far. It is currently believed that antioxidant properties of catechin arise from the stability of the stable quinone formed in the catechol (3,4-dihydroxybenzene) group on the B ring, Scheme 3, which participates in the delocalization and stabilization of the corresponding antioxidant radical [18]. The -OH group on the C ring and those located on the A ring (resorcinol group), do not contribute substantially to their antioxidant efficiency but only increase its water solubility by adding polar groups to the molecule.



Scheme 3. Oxidation mechanism of CAT to yield a stable quinone [19].

Determining the partition constants of catechin: application of the pseudophase kinetic model.

Previous solubility experiments [20] showed that CAT is oil-insoluble and thus, only distributes between the aqueous and interfacial regions of emulsions, Scheme 4. The solvent properties of the interfacial region are different from those of the aqueous region and, consequently, their solubility in those regions is different. Determining the solubility of CAT in the aqueous region is a relatively simple task by employing a number of analytical methods [20, 21]. However, determining the solubility in the interfacial region is quite complex if not impossible because the interfacial region cannot be isolated from the aqueous and oil regions whithout disrupting the exisiting equilibria, and because the interfacial region is a highly anisotropic region whose exact composition is unkown.



Scheme 4. Illustration of the partitioning of CAT between the interfacial and aqueous regions of an emulsion. Φ_I indicates the volume fraction of the surfactant, P_W^I, is the partition constant between aqueous and interfacial region and k_I is the rate constant for reaction between 16-ArN_2^+ and CAT in the interfacial region.

Rather than determining its solubility, we determined the partition constant P_w^I (i.e., its distribution, equation (3) by employing a well-established kinetic method, based on the reaction between a hydrophobic arenediazonium ion, 16-ArN_2^+, and the antioxidant in the intact emulsion. The method is described in detail elsewhere and only a brief description will be given here [14]. Experimentally, we determine the variation of the observed rate constant, k_{obs}, with the surfactant volume fraction Φ_I (defined as $\Phi_I = V_{surf}/V_{emulsion}$), and the relationship between P_W^I and the observed rate constant k_{obs} can be established on the grounds of the pseudophase kinetic model, equation (4). In brief, for a bimolecular reaction in an emulsion, the observed rate, v, is the sum of the rates in each region of the macroemulsion[14]. The reaction between 16-ArN_2^+ and CAT takes place exclusively in the interfacial region of the emulsion because 16-ArN_2^+ is water insoluble due to its long hydrophobic tail, and is oil insoluble because of its cationic headgroup, Scheme 4.

The mathematical relationship between k_{obs} and P_W^I, equation (4), has been derived elsewhere[14]. The reciprocal form of equation (4), equation (5), predicts that plots of 1/ k_{obs} *vs* Φ_I should be linear with positive intercepts, from where P_W^I values can be obtained.

$$P_W^I = \frac{(CAT_I)}{(CAT_W)} \tag{3}$$

$$k_{obs} = \frac{k_I [CAT_T] P_W^I}{\Phi_I P_W^I + \Phi_W} \tag{4}$$

$$\frac{1}{k_{obs}} = \frac{\Phi_W}{k_I [CAT_T] P_W^I} + \frac{\Phi_I}{k_I [CAT_T]} \tag{5}$$

Once P_W^I is known, determining the percentage of the antioxidant the water and interfacial regions is straightforward by employing equations (6) and (7).

$$\%CAT_W = \frac{100\Phi_W}{\Phi_I P_W^I + \Phi_W} \tag{6}$$

$$\%CAT_I = \frac{100\Phi_I P_W^I}{\Phi_I P_W^I + \Phi_W} \quad (7)$$

The aqueous and interfacial concentrations of CAT can be obtained from equations (8) and (9), respectively.

$$(CAT_W) = \frac{\%CAT_W [CAT_T]}{\Phi_W} \quad (8)$$

$$(CAT_I) = \frac{\%CAT_I [CAT_T]}{\Phi_I} \quad (9)$$

EXPERIMENTAL

Materials

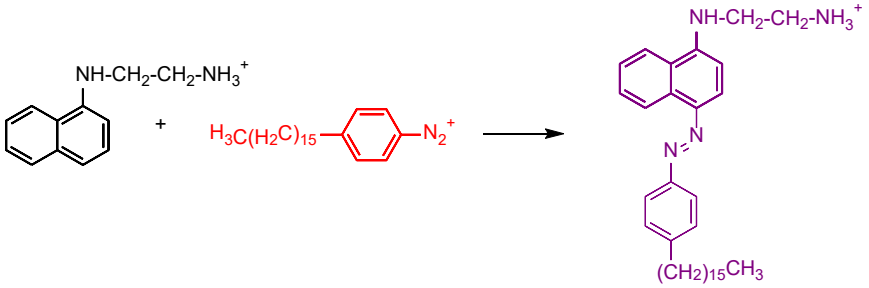
Catechin (Aldrich), the polyoxyethylene (20) sorbitan monolaurate emulsifier (Tween 20, Fluka) and the corn oil (Across Organics, d = 0,918 g/mL) stripped from endogenous antioxidants were of the highest purity available and used as received. 4-Hexadecylbenzene diazonium tetrafluoroborate, 16-ArN_2BF_4, was prepared as described elsewhere [14] and was stored in the dark at low temperature to minimize its descomposition. All aqueous acid solutions were prepared by employing citric acid-sodium citrate buffer (pH = 2.14, 0.04 M). Solutions of the coupling reagent N-(1-naphthyl) ethylenediamine (NED, Aldrich) were prepared in a 50:50 (v/v) BuOH:EtOH mixture to finally give [NED] = 0.02 M.

Emulsion preparation

Corn oil-in-water emulsions were prepared by mixing 4 mL of stripped corn oil and 6 mL of a citric-citrate buffer solution (pH 2.14) containing a weighed amount of non-ionic surfactant volume fraction of emulsifier, Φ_I. The volume fraction of emulsifier was varied from $\Phi_I = 0.005$ to $\Phi_I = 0.04$. CAT was dissolved in the buffered aqueous solution employed to prepare the emulsions. The stoichiometric concentration of CAT in the emulsion was $[CAT_T] = 4$ mM. The oil and aqueous mixture was stirred with a high-speed rotor (Polytron PT 1600 E) for 1 minute and transferred to a continuously stirred thermostated cell. No phase separation was visually (naked eye) observed within 3-4 hours, a time much higher than that required to monitor the reaction between 16-ArN_2^+ and CAT for more than 3-4 half-lives.

Methods

Determining k_{obs} at different surfactant volume fractions. The observed rate constants, k_{obs}, for the reaction between the chemical probe 16-ArN_2^+ and CAT were determined as in previous work by employing a derivatization method [9-11, 14] that exploits the rapid reaction between 16-ArN_2^+ and the coupling agent N-(1-naphthyl)ethylenediamine, NED. The reaction leads to the formation of a stable azo dye, Scheme 5, whose absorbance, Figure 2, can be measured by UV-Vis spectroscopy. Details can be found in detail elsewhere [22].



Scheme 5. Reaction between the coupling agent N-(1-naphthyl) ethylenediamine, NED, with 4-hexadecylbenzenediazonium ions, leading to the formation of an azo dye.

In a typical experiment, the reaction between the 16-ArN_2^+ and the catechin was initiated by adding an aliquot (16 µL) of a 0.17M stock 16-ArN_2^+ solution in acetonitrile to a thermostated and continuously stirred emulsion. At selected times, aliquots (200 µL) of emulsion were transferred to vessels containing 2.5 mL of a 0.02M alcoholic solution 50:50 (v:v) BuOH/EtOH- mixture of NED. Under our experimental conditions, 16-ArN_2^+ reacts with NED much faster than with CAT so that the reaction between 16-ArN_2^+ and CAT is effectively quenched. Values of k_{obs} were determined from the variations of the absorbance at $\lambda = 572$ nm with time by fitting the pairs of data (absorbance, time) to the first-order equation (10) by employing a nonlinear least-squares method provided by a commercial computer program (GraFit 5.0.5). In equation (10), A_t, A_o and A_∞ are measured absorbance at any time, at t = 0 and at infinite time.

$$\ln(A_t - A_\infty) = -k_{obs}t + \ln(A_0 - A_\infty) \quad (10)$$

Figure 2 is illustrative and shows a typical variation of the absorbance of the azo dye with time and the corresponding linear plot according to equation (10).

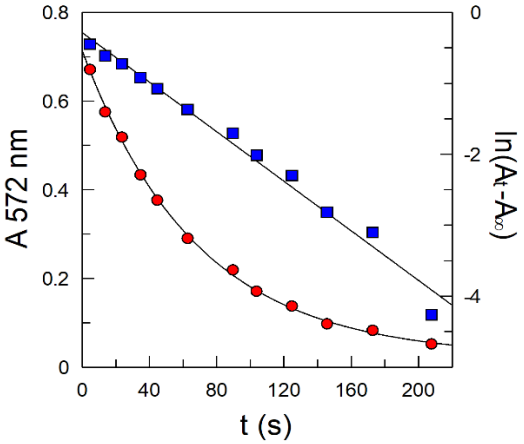


Figure 2. Variation in absorbance of the formed azo dye (-●-) and ln[A_t-A_∞] (-■-) plots for the reaction of 16-ArN_2^+ with CAT in 4:6 (O/W) corn oil-in-water emulsions (pH 2.14) and Tween 20. [CAT] ≈ 4 mM, [16-ArN_2^+] = 0.29 mM, [NED] = 0.02M, T = 25ºC.

Statistical analysis

Duplicate or triplicate experiments gave k_{obs} values within ± 7-9 %. Data are displayed as means ± standard deviation of the measurements.

RESULTS AND DISCUSSION

Partition constants of CAT in corn oil-in-water emulsions: effects of temperature and determination of thermodynamic parameters

The partition constants of CAT were determined, as indicated before, from the variation of k_{obs} with Φ_I at different temperatures (T = 15 - 35ºC) in 4:6 corn oil-in-water emulsions (pH = 2.14), Figure 3, by fitting the experimental data to equations (4)-(5). Figure 3 is representative and shows that, at T = 15º and 25 ºC, k_{obs} decreases 1-3-fold on going from Φ_I = 0.005 to Φ_I = 0.04, consistent with the predictions of equation (4). These variations are quite similar to those found when analysing the behaviour of other antioxidants such as caffeic acid or hydroxytyrosol [9, 11].

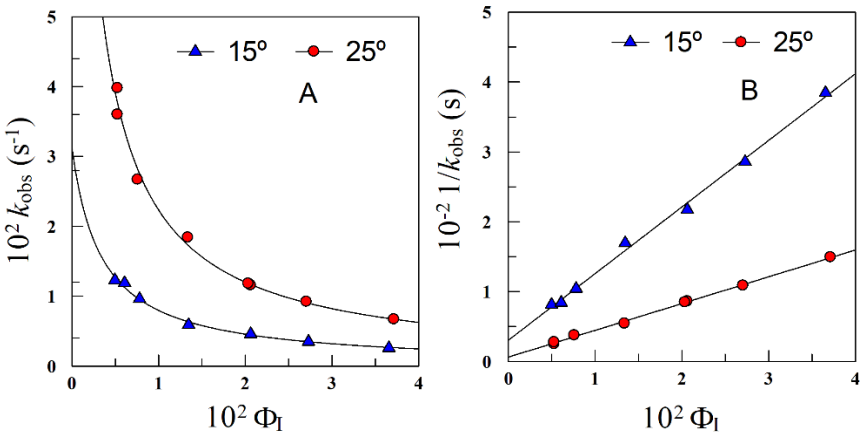


Figure 3. Effects of increasing Φ_I on k_{obs} **(A)** and on $1/k_{obs}$ **(B)** for the reaction of 16-ArN_2^+ with catechin at different temperatures. The solid lines are the theoretical curves obtained by fitting the experimental (k_{obs}, Φ_I) or ($1/k_{obs}$, Φ_I) pairs of data to equations 4 and 5, respectively. Experimental conditions: 4:6 corn oil-in-water emulsions stabilized with Tween 20. [16-ArN_2^+] = 0.29 mM, [CAT] = 4mM, pH 2.14 (citric-citrate buffer 0.04M).

The straight lines shown in Fig. 3B were used to obtain the partition constant P_W^I. Table 1 shows that P_W^I increases 1.6 - 4.3-fold upon increasing T from 15 to 35 ºC.

As we have shown in previous works [23, 24], the transfer of molecules between the different regions of fluid emulsions is not rate limiting and thus, the system is in dynamic equilibrium. This means that the chemical potential of CAT in each region, defined by equations (11)-(12), should be identical. In equations (11)-(12), $\mu_{CAT}^{0,W}$ and $\mu_{CAT}^{0,I}$ are the standard chemical potential, and X_{CAT}^{W} and X_{CAT}^{I} are the mole fractions of catechin in the aqueous and interfacial regions, respectively.

Table 1. Values of the partition constant P_W^I for CAT in corn oil-in-water emulsions at different temperatures.

T(ºC)	P_W^I
15	190
20	310
25	360
35	804

$$\mu_{CAT}^{W} = \mu_{CAT}^{0,W} + RT \ln X_{CAT}^{W} \text{ (aqueous)} \quad (11)$$

$$\mu_{CAT}^{I} = \mu_{CAT}^{0,I} + RT \ln X_{CAT}^{I} \text{ (interfacial)} \quad (12)$$

The Gibbs free energy, $\Delta G_T^{0,W\to I}$, for the transfer of 1 mol of catechin from the aqueous to the interfacial region, is given by equation (13), where V_m^W and V_m^I are the molar volumes of water and emulsifier. Values for the molar volumes can be obtained from literature density values and we assume that they are essentially constant over the relatively small temperature ranges employed (T ≈ 290 - 310 K). Thus, $\Delta G_T^{0,W\to I}$ is an easily accessible parameter that can be determined at a given temperature from the partition constant values in Table 1.

$$\Delta G_T^{0W\to I} = \mu_{CAT}^{0,I} - \mu_{CAT}^{0,W} = RT \ln \frac{V_m^W}{P_W^I V_m^I} \quad (13)$$

$$\Delta H_T^{0,W\to I} = R \left[\frac{\partial \left(\ln P_W^I \right)}{\partial \left(\frac{1}{T} \right)} \right]_P \quad (14)$$

The values of P_W^I in Table 1 can be also employed to obtain the enthalpy of transfer, $\Delta H_T^{0,W\to I}$, by using the van't Hoff, equation (14) which predicts that a plot of ln P_W^I with 1/T should be a straight line. Figure 4 shows that this prediction is fulfilled, and from the slope of the straight line, a value for $\Delta H_T^{0,W\to I}$ can be obtained. The entropy for the transfer of CAT from the aqueous to the interfacial region, $\Delta S_T^{0,W\to I}$, can be obtained by using the Gibbs equation (15).

$$\Delta S_T^{0,W\to I} = \frac{\Delta H_T^{0,W\to I} - \Delta G_T^{0,W\to I}}{T} \quad (15)$$

The thermodynamic parameters for CAT transfer were obtained from equations (13)-(15) and they are listed in Table 2.

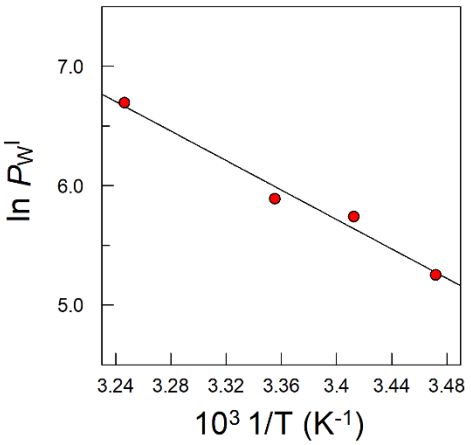


Figure 4. Plot of the variation of ln P_W^I *versus* 1/T according to the van´t Hoff equation (11). Data extracted from Table 1.

Table 2. Thermodynamic values (Gibbs free energy, enthalpy and entropy) for the transfer of 1 mol of CAT from the aqueous to the interfacial region of corn oil-in-water emulsions.

$\Delta G_T^{0W\to I}$ (kJ/mol)	$\Delta H_T^{0W\to I}$ (kJ/mol)	$\Delta S_T^{0W\to I}$ (kJ/mol K)
-24.81	-51.29	-0.09

Results suggest that the transfer of CAT from the aqueous to the interfacial region is spontaneous at any T because $\Delta G_T^{0W\to I}$ is negative. $\Delta S_T^{0W\to I}$ is also negative, suggesting that there is not a net increase in disorder of the transfer process of CAT from the aqueous region to the interfacial region of the emulsions. The $-T\Delta S_T^{0W\to I}$ contribution is thus positive, however, the negative entalphy contribution is much higher than the $-T\Delta S_T^{0W\to I}$ contribution, therefore suggesting that the transfer of catechin from aqueous to interfacial region is essentially an entalphy-driven process.

Interfacial concentrations of CAT in corn oil emulsions: effects of temperature, surfactant concentration and oil-to-water ratio

The determined P_W^I values, Table 1, are much higher than the unit, ranging 190 – 804, suggesting that catechin is mostly located in the interfacial region of the emulsion. The value at T = 25 ºC, P_W^I = 360, is much higher than those obtained for hydrophilic catecholics such as hydroxytyrosol [11] (P_W^I = 120) but similar to that of caffeic acid [9] (P_W^I = 370). All together, these results show that the partition constants of catecholics cannot be predicted exclusively on the basis of their polarity [9-11] and need to be determined for each species

in the intact emulsions. Moreover, the negative $\Delta H_T^{0W\to I}$ value shows that van der Waals interactions and hydrogen bonds between -OH groups of catechin and polyoxyethylene groups of the surfactant may play a major role in the the transfer of catechin from the aqueous to the interfacial region. This also may have important consequences on the effective concentration of CAT whose values in the aqueous and interfacial concentrations can be determined by employing equations (8) and (9), respectively. The results also suggest that the presence of polyoxiethylene sorbitan fatty acid esters (Tween 20) and polar fatty acid of corn oil in the interfacial region contribute to enhance the interactions with -OH group of catechin at high temperatures.

Figure 5 shows the variation of the local aqueous and interfacial concentrations of catechin with the surfactant volume fraction in the T = 15 – 35 ºC range.

At any given temperature, the effective interfacial concentrations of CAT are 20 -200 times higher than the stoichiometric concentration ($[CAT_T] = 4\times10^{-3}$ M), depending on the temperature and, mainly, on the surfactant concentration. On the contrary, the effective aqueous concentration of CAT is much lower than the stoichiometric concentration. This means that the interfacial region of the emulsion acts as a very efficient "microreactor" concentrating the reactant (in this case, the antioxidant) and thus increasing notably its efficiency in inhibiting the oxidation of lipids with respect to a bulk system. Note that an increase in the surfactant volume fraction Φ_I decreases the effective interfacial concentration because of the increase in the interfacial volume.

Results shown before suggested that the transfer of CAT from aqueous to interfacial region is entalphy driven. Enthalpic contributions are usually associated to the strength of hydrogen bonds and this contribution may change in a different extent in the aqueous and interfacial regions upon increasing temperature because of the increase in the thermal motion of the catechin. Thus, upon increasing the temperature, the local concentrations of CAT can change.

Figure 6 shows the effects of increasing temperature on the effective concentrations of CAT in the aqueous and interfacial region of the corn emulsions at selected surfactant volume fractions. Upon increasing T, the effective concentration of CAT decreases in the aqueous region, increasing concomitantly that in the interfacial region. Note the effects of the surfactant volume fraction. At low $\Phi_I = 0.005$ the effective interfacial concentrations increases a modest 1.5-fold, meanwhile at $\Phi_I = 0.04$ the increase is negligible.

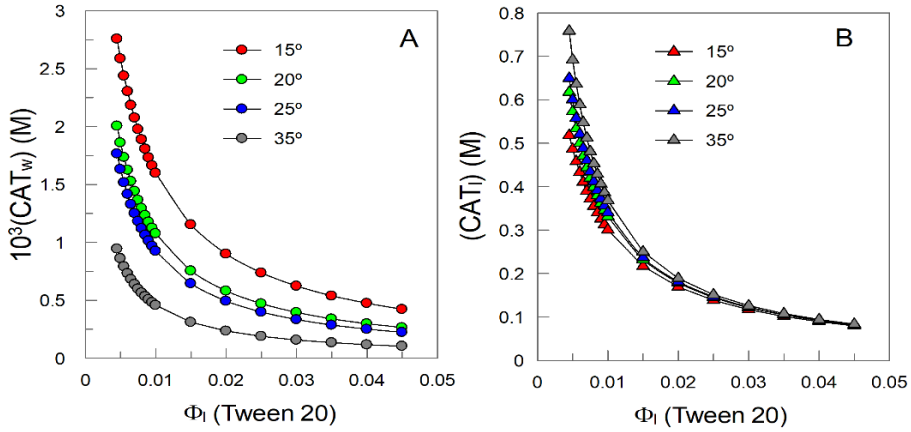


Figure 5. Variation of the effective concentration of CAT in the aqueous **(A)** and interfacial **(B)** regions of 4:6 corn oil emulsions as a function of temperature and emulsifier concentration. The effective concentrations (in parentheses) are expressed as moles of antioxidant per volume of the particular region, meanwhile stoichiometric concentrations are defined in terms of moles of the antioxidant per volume of the emulsion.

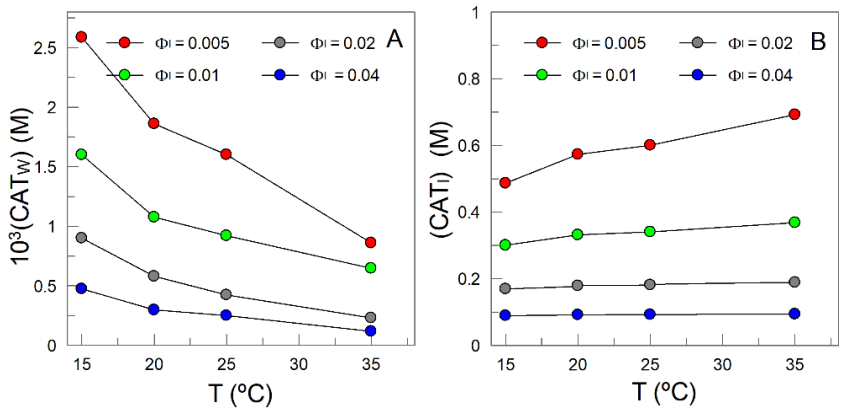


Figure 6. Effect of temperature on the concentration of catechin in the aqueous **(A)** and interfacial **(B)** regions of 4:6 corn oil-in-water emulsions at selected Tween 20 volume fractions.

Figure 6 shows that the compostion of the interfacial region alters the balance of the various intra- and intermolecular forces that define the actual distribution of the antioxidant. Thus, one could also expect that the oil-to-water ratio may also affect the effective concentrations of the antioxidant. Figure 7 shows the variation in the effective aqueous and interfacial concentrations of CAT upon increasing the oil volume fraction Φ_O (defined as $\Phi_O = V_{oil} / V_{emulsion}$). At any temperature, the interfacial concentrations of CAT increase upon increasing Φ_O, suggesting that its solubility is higher in the interfacial region than in the aqueous region.

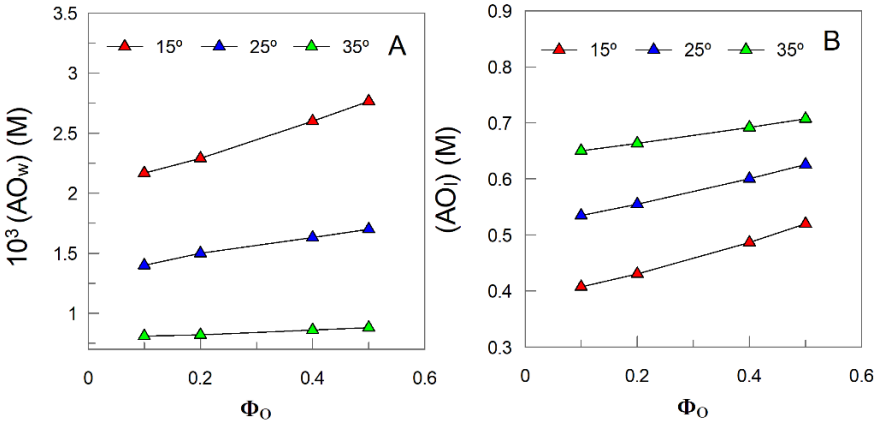


Figure 7. Effect of oil volume fraction (Φ_O) on the concentration of catechin in the aqueous **(A)** and interfacial **(B)** regions of 4:6 corn oil-in-water emulsions at different temperatures.

CONCLUSIONS

We have evaluated the effects of temperature, oil- to-water ratio and surfactant concentration on the effective concentrations of a model flavonoid in the aqueous and interfacial regions regions of corn oil-in-water emulsions. The Gibbs free energy, enthalpy and entropy for the transfer of 1 mol of CAT from the aqueous to the interfacial region of the emulsions were also evaluated.

P_W^I values were obtained by employing a kinetic method, and the good straight line obtained for the variation of ln P_W^I with 1/T (Van´t Hoff equation), Figure 4, demonstrates the feasibility of our methodology to determine valuable thermodynamic parameters for the transfer of antioxidants from the aqueous (or oil) regions to the interfacial region of emulsions. The method also allows determining the effective concentrations of the antioxidants in those regions, which are basic to rationalize the antioxidant efficiency of emulsions in multiphasic systems. At present, no other methodology allows estimations of these parameters because reported methods require the rupture of the emulsion, disrupting the existing equilibria.

Results show that the transfer of CAT from the aqueous to the interfacial region is spontaneous and enthalpy driven. The sensitivity of the changes in the P_W^I values with T is consistent with the significant changes in the solvation properties on going from an aqueous to interfacial region. The changes in P_W^I values with T contrast strongly with

the modest dependence of P_W^I obtained for other hydrophilic antioxidants such as gallic or caffeic acid [23, 24].

The effects of surfactant concentration, oil-to-water ratio and temperature on the concentration of CAT in the aqueous and interfacial regions of corn oil-in-water emulsions were evaluated. Our results show that the larger variations in the interfacial concentrations are obtained when changing the surfactant concentration, meanwhile changes in the O/W ratio and T (other things being equal) only have a modest effect.

Finally, we would like to stress the importance of determining the effective concentrations of antioxidants in the interfacial region of emulsions because, in general, the antioxidant efficiency depends on such values, the higher the interfacial concentration, the higher is the antioxidant efficiency.

Acknowledgement: *Finacial support from the following institutions is acknowledged: Xunta de Galicia (REDUSO, Grant number ED431D 2017/18), and University of Vigo. S. L - B thanks University of Vigo for a post-doctoral research contract (Talent Recruitment Program, 2018)*

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